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Molecular epidemiological aspects of bovine tropical theileriosis in cattle in and around Patna, Bihar, India

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Abstract

T. annulata is the principal causal agent of Bovine Tropical Theileriosis in India, posing a threat to animal health and productivity. The present investigation was carried out to record the molecular prevalence of Bovine Tropical Theileriosis in and around district of Patna, Bihar. A total of 200 blood samples were collected from cattle presented with clinical signs suggestive of theileriosis. The PCR based screening using the genus specific primers of *18S rRNA* gene detected 120 samples (60.00%) positive for *Theileria* spp. The species specific primers of *cyto1* gene detected 77 samples (38.50%) positive for *Theileria annulata* which included 12 samples found negative by Giemsa staining. Significantly, highest prevalence of disease was recorded in cattle above 2 years of age, in cross bred cattle, and in monsoon season.

Keywords: Bovine tropical theileriosis, prevalence, PCR

Introduction

Theileriosis in cattle is caused by the apicomplexan parasite *Theileria* spp., which is a major tick-borne disease. *T. annulata* is the principal causal agent in India, posing a threat to animal health and productivity (Sudan *et al.*, 2012)^[30]. In India, an economic loss of \$800 million has been reported as a result of *T. annulata* infection (Brown, 1997)^[8]. Diagnosis of Bovine theileriosis mainly relies upon clinical signs, history of tick infestation on body, and demonstration of parasitic forms in Giemsa stained blood smears. Microscopy has several drawbacks, including the fact that it is time-consuming, labor-intensive, and misleading due to morphological similarities with other parasites, as well as it requires a skilled technician (Criado-Fornelio, 2007^[10] and Mans *et al.*, 2015^[23]). To overcome for such limitations of traditional microscopic examination, PCR-based assays allow parasite identification at levels considerably below the detection limit of routinely employed parasitological techniques, and thus recommended as the best diagnostic tool for the detection of *T. annulata* in epidemiological studies due to its high sensitivity and specificity (Almería *et al.*, 2001)^[2]. Moreover, it has superiority in detecting parasitic infections associated with clinical signs (clinical form) and the infection without clinical signs (subclinical form) (d'Oliveira *et al.*, 1995^[11] and Almería *et al.*, 2001^[2]). The disease, which is hard to eliminate fully, is linked to regional and seasonal epidemics. Changes in climatic conditions over the last few decades have favoured tick multiplication and, as a result, a progressive increase in the incidence of tick-borne disease (Kohli *et al.*, 2014)^[20]. Theileriosis susceptibility has increased as a result of increased cattle population and the introduction of exotic/crossbred cattle, particularly in endemic areas.

The present investigation was carried out to record the molecular prevalence of Bovine Tropical Theileriosis in and around district of Patna, Bihar state over a period of two year from March 2020 to February, 2022. The prevalence of the disease was determined age wise, sex wise, breed wise and season wise.

Materials and Methods

The cattle presented with any or combined clinical signs suggestive of theileriosis like high fever (104-107°F), swelling of submandibular and subscapular lymph nodes, pale mucous membrane, suspended rumination, bilateral nasal discharge, lacrimation and or which have the problem of tick infestation etc. were included in the study. A total of 200 blood samples were

collected from the jugular vein of each animal into EDTA coated vial. Genomic DNA from each blood sample was isolated using the Wizard® Genomic DNA Purification Kit (Promega, USA) following the manufacturer instructions with slight modifications, and then stored at -20 °C until future use. *Theileria* genus specific 18S rRNA gene primers targeting the amplicon of 778 bp and *Theileria* species specific *cyto1* gene primers targeting the amplicon of 312 bp were used for PCR assay.

Statistical analysis

Chi square test was applied to evaluate the significant differences ($P < 0.05$) among age, sex, breed and season.

Results and Discussion

Patna is located on the southern bank of the river Ganges. The city is bounded in the north by river Ganga, in the south and east by river Punpun and in the west by river Sone. Patna district is situated between 25°13' and 25°45' North latitude and 84° 43' and 86° 44' East longitude with a height of 51 meters above mean sea level. Patna has a humid subtropical climate with extremely hot summers from late March to early June, the monsoon rainfall from late June to September and a mild winter from November to February (Singh and Singh, 2017) [29].

Out of 200 samples tested, 120 samples were recorded positive to *Theileria* spp. through PCR indicating the overall prevalence of bovine theileriosis to a magnitude of 60% (120/200) (Table 1). The samples positive for *Theileria* spp. were subjected to *T. annulata* specific primer revealed overall prevalence of 38.50% (77/200) for *T. annulata* and rest 21.50% (43/200) samples which were positive by *Theileria* genus specific primer did not amplify for *T. annulata* on employment of the *Theileria* species specific primer set indicating for the presence of other benign *Theileria* spp. (Table 1).

The overall species wise prevalence of tropical theileriosis in the present study was in close agreement with the findings of Almeria *et al.* (2001) [2] and Madhukar *et al.* (2016) [21], who reported 41.3% and 41.7% of *Theileria annulata*. The variations in overall occurrence in theileriosis in cattle might due to distribution and density of the vectors, geo-climatic conditions, season, breed, and age of the animals.

Age wise molecular prevalence of *T. annulata*

The disease occurrence pattern was found in different age groups. The highest prevalence of 39.66% (71/179) was recorded in animals above two years of age i.e. in adult age group, and the least 28.57% (6/21) prevalence was recorded in below 2 years of age (Table 2). The findings are in close agreement with the observations of Farooq *et al.* (2019) [14], who recorded the highest incidence in >2 years age groups, followed by age groups of 6 months to 2 years and 0-6 months. Similar observations were made by Ananda *et al.* (2009) [3] in cross bred cattle in Bangalore North, who observed infection among the age groups of 4-6 yrs., 1-2 yrs., and < 6 months. Raina *et al.* (2005) [25] reported high prevalence of haemoprotozoan diseases in adult animals compared to calves in bovines in Jammu district. The lesser incidence of theileriosis in the animals of young age group could be due to more attention paid by the farmers in providing proper housing, intensive feeding and prevention from ticks (Bhutto *et al.*, 2010) [6]. The presence of maternal antibodies in young animals might be another cause for lower

infection in young animals. Haque *et al.* (2022) [17] who reported the lowest prevalence of theileriosis in young animals which might be due to innate and acquired immunity. The higher incidence of theileriosis with an increase in the age of the animals could be due to the grazing of animals in pasture land where they are more prone to be infested by the vector ticks.

Sex wise molecular prevalence of *T. annulata*

Studies on the sex wise occurrence of theileriosis revealed a non-significant ($P > 0.05$) difference in between male and female cattle, but higher rate of prevalence was noticed in female cattle (76/193 i.e., 39.37%) than those of male (1 / 7 i.e., 14.28%) cattle (Table 2). The findings of higher prevalence of tropical theileriosis in females as compared to males are in agreement with the results of Durrani *et al.* (2008) [13], Khan *et al.* (2017) [20] and Brahmhat *et al.* (2019) [7]. Usually, the female cattle are in persistent physiological stress due to longer milk production and breeding, this stress along with suppression of immunity may predispose them to higher risk of getting infected with Bovine Tropical Theileriosis (Bhangale *et al.* 2021) [4]. Moreover, the dairy farmers especially prefer to raise female calves as replacement stock and are less interested in rearing on male calves, hence there were less male calves presented for diagnosis.

Breed wise molecular prevalence of *T. annulata*

Studies on the breed wise occurrence of theileriosis revealed a significantly ($P < 0.05$) higher prevalence rate in cross bred cattle (73/174 or 41.95%) than those of indigenous (4/26 or 15.38%) cattle (Table 2). Similar observations were made by earlier researchers (Singh *et al.*, 2001 [28]; Panda *et al.*, 2011 [24]; Acharya *et al.*, 2016 [1] and Ghosh *et al.*, 2018) [15] for higher occurrence of theileriosis in exotic and crossbred cattle than indigenous breeds of cattle due to their high susceptibility to the disease. The variation in the susceptibility is due to a variation in the immune response to create pro-inflammatory cytokines, which is higher in exotic animals or native breeds harbouring specific genes for tolerance (Jensen *et al.*, 2008 [18] and Glass *et al.*, 2012 [16]).

Season wise molecular prevalence

Season wise, a significantly ($p < 0.05$) higher rate of theileria infections was observed in monsoon 49.50% (50/101) as compared to summer 31.42% (22/70) and winter 17.24% (5/29) (Table 2). The findings of the present study were in accordance with the observations made by Roy *et al.* (2004) [26], Panda *et al.* (2011) [24], Kohli *et al.* (2014) [20], Bhatnagar *et al.* (2015) [5] and Maharana *et al.* (2016) [22]. They found highest prevalence in monsoon season. This might be due to more number of ticks in monsoon which were developed during summer months (Ananda *et al.*, 2009) [3], and played a significant role in propagation and multiplication of haemoparasites in the vectors and hosts during monsoon season (Ghosh *et al.*, 2018) [15]. Debbarma *et al.* (2017) [12] reported the highest incidence of *Hyalomma* sp. in monsoon, which is responsible for transmission of *T. annulata* in cattle. In contrast to present study, Shekhar and Haque (2007) [27] and Chaudhri *et al.* (2013) [9], reported maximum incidence in summer followed by rainy season, minimum in winter and they stated that the disease was related to hot and humid climate and higher infectivity of vector ticks.

Table 1: Animals positive for the presence of *Theileria* sp. by microscopic examination (ME) of blood smears and by PCR for *Theileria* spp. and *Theileria annulata*

Technique	Animal Screened	Positive for theileriosis	Percent positive (%)
Microscopic Examination of <i>Theileria</i> spp.	200	65	32.50
<i>Theileria</i> spp. by PCR	200	120	60.00
<i>T. annulata</i> by PCR	200	77	38.50
<i>Theileria</i> spp. other than <i>T. annulata</i>	200	43	21.50

Table 2: Age, Sex, Breed and Season wise statistical analysis of prevalence of Bovine tropical theileriosis in cattle by PCR

Factors	Category	No. of Sample Screened	No of sample Positive	Chi Square	Degree of freedom	p-value
Age	Below 2 Yrs.	21	6(28.57%)	6.276	1	0.012*
	Above 2 Yrs.	179	71(39.66%)			
Sex	Male	7	1(14.28%)	1.796	1	0.180 ^{NS}
	Female	193	76(39.37%)			
Breed	Indigenous	26	4 (15.38%)	6.744	1	0.009*
	Crossbred	174	73(41.95%)			
Season	Monsoon	101	50(49.5%)	12.0180	2	0.002*
	Winter	29	5(17.24%)			
	Summer	70	22(31.42%)			

*Significant at $p < 0.05$, NS-Non-significant at $p > 0.05$

Conclusion

It might be concluded that overall prevalence of *T. annulata* was recorded to be 38.50% based on using species specific *cyto1* gene primers by PCR and rest 21.50% (43/200) samples which were positive by *Theileria* genus specific primer did not amplify for *T. annulata* on employment of the *Theileria* species specific primer set indicating for the presence of other benign *Theileria* spp. So, there is need of further screening for some other benign *Theileria* spp. The highest prevalence of disease was recorded in cattle above 2 years of age, in cross bred cattle, and in monsoon season.

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